American Society of Cytopathology (ASC) and Papanicolaou Society of Cytopathology (PSC)
Joint Position Statement on
The Use of Molecular Testing on Cytologic Specimens

Cytomorphologic evaluation of exfoliative and aspiration cytology specimens play an essential role in guiding the management of patients undergoing evaluation for neoplasia. In recent years, medicine has witnessed an explosive growth in our recognition and understanding of molecular genetic abnormalities that drive the development and progression of various cancers, as well as the therapeutic relevance of these abnormalities which mandates that molecular testing be performed for management decisions. Technologic advances that enable minimally invasive interventional modalities to obtain tissue for diagnosis have resulted in the increasing importance of cytologic specimens for patient management. Therefore, judicious and effective integration of molecular ancillary testing with cytopathology specimen acquisition, processing, and analysis is required in order to ensure timely and appropriate patient management.

Molecular studies can serve as diagnostic adjuncts to aid in cytomorphologic classification and/or to interrogate specific relevant biomarkers, thereby providing information pertinent to prognosis and targeted therapy. Salient examples illustrating the intimate interface between cytopathology and molecular diagnostics include: (1) human papillomavirus testing on cervical cytology specimens and metastatic and primary head and neck cancers; (2) mutation panel and gene expression classifier testing on indeterminate thyroid fine needle aspirates (FNAs); (3) *EGFR/ALK/ROS1* analysis on advanced-stage lung adenocarcinoma; (4) *BRAF* mutational analysis in melanoma and thyroid cancer FNAs; (5) fluorescence in-situ hybridization (FISH) testing applied to a variety of specimens such as urinary tract cytology, pancreatobiliary brushings, effusions, and cytologic samples from sarcomas and lymphomas; and (6) the application of next generation sequencing (NGS) to cytologic specimens.

The current era of personalized “precision medicine” continues to evolve at an accelerated pace. Thus, effective multidisciplinary collaboration and coordination amongst cytopathologists, surgical pathologists, molecular pathologists, clinicians, cytotechnologists, and laboratory staff becomes even more essential. The ultimate goal of these multidisciplinary efforts is to devise “best practice” approaches for cytologic specimen acquisition and processing. This would ensure that both cytomorphologic evaluation and ancillary testing can effectively be performed, with selection of appropriate molecular tests and avoidance of inappropriate and unnecessary testing. In the setting of escalating health care costs associated with laboratory testing as well as targeted therapy, the judicious and effective use of molecular tests is paramount.

Cytopathology laboratories utilize myriad methodologies to process various cytologic specimen types. Given the versatility of cytopreparatory techniques, the development and careful validation of molecular assays for these platforms is important. Molecular diagnostic laboratories are increasingly validating molecular diagnostic assays on cytopreparatory techniques, such as direct smears, cell blocks, liquid-based preparations, cytospins, cell pellets, and touch imprints. Archived slide preparations, such as direct smears, also represent a valuable, robust source of material for the performance of these tests. It is important to note that many cytopreparatory techniques can be performed in the absence of formalin fixation. Formalin fixation leads to the crosslinking of nucleic acids and proteins, fragmentation of isolated nucleic acid material, and the possibility of sequence alterations.

FNA samples can offer an advantage over core needle biopsies as they often enable the acquisition of a naturally enriched tumor cell population with respect to stromal elements. FNAs also allow for appraisal of multiple wide areas of a target lesion. Coupled with the utilization of rapid on-site evaluation (ROSE) and confirmation of adequacy, cytopathologists and cytotechnologists are uniquely prepared to provide a real-time impact on FNA specimen procurement, by identifying pertinent molecular diagnostic assays relevant to a patient's medical care, triaging the specimen appropriately for necessary molecular tests, and engaging in collaboration with clinical colleagues.

A chief concern raised regarding the use of cytologic specimens surrounds the quantitative adequacy of material. Many molecular tests, especially those that are PCR based, utilize large degrees of target amplification thereby allowing for testing on extremely small quantities of material. For instance, based on the assumption that a diploid cell contains 6 picograms of DNA, PCR based assays requiring only 1 nanogram of input DNA equates to approximately 166 intact diploid cells. As an illustration, a recent report demonstrated that cytologic samples of lung adenocarcinoma containing as low as 100-199 tumor cells could be effectively utilized for *EGFR* mutational analysis. Ultimately, despite variations in the quantity of cellular sample obtained, the reliability of any molecular test rests on its analytic sensitivity defined as the minimal percentage of tumor cells/mutant alleles required to detect the molecular alteration in question. Effective modalities to assess for DNA mutations in cytologic samples include Sanger sequencing, pyrosequencing, allele-specific PCR fragment analysis, and NGS.

Cytologic specimens are also adequate for FISH analysis as this can be typically carried out on as few as 50 to 100 cells of interest. FISH testing can be used to examine chromosome gains or losses as exemplified by FISH analysis of urinary tract cytology and pancreatobiliary brushings. Alternatively, FISH testing can be used to interrogate for the presence or absence of chromosomal translocations using locus specific probes. Depending on the design and

validation of a given FISH assay, either whole nuclei (represented on direct smears, ThinPrep slides, cytospins, and touch preparations) or sectioned nuclei (cell blocks) can be effectively utilized.

Reverse transcriptase-polymerase chain reaction (RT-PCR), utilized to assess for the presence of chimeric mRNA fusion transcripts, can be effectively performed on cytologic preparations. RNA integrity - influenced by variables such as ischemia time, transport circumstances, type and length of fixation, processing and storage conditions - is crucial for the successful performance of RT-PCR assays. RT-PCR testing can be performed on formalin-fixed paraffin embedded (FFPE) or alcohol-fixed cell block preparations. Furthermore, studies have shown that cytologic direct smears are amenable for RT-PCR assays.

NGS is emerging as a powerful and sensitive tool to interrogate the mutational status of multiple genes simultaneously with only nanogram levels of nucleic acid material. Recent studies have demonstrated that cytologic direct smears and cell blocks from FNA samples can be effectively utilized for NGS. The sequence information is comprehensive and statistically indistinguishable from that obtained from paired FFPE histologic samples.

In the current and evolving era of precision medicine, Cytopathology is at the forefront of tissue procurement. Cytologic specimens represent a useful and valuable source of cellular material for the evaluation of various molecular diagnostic tests that contribute to optimal patient care. Traditional ROSE for adequacy assessment and cytomorphologic review along with immunocytochemical evaluation, when necessary, represents a fundamental foundation. Ultimately, the most powerful molecular study for any individual case is one in which molecular information is well integrated with the cytomorphologic interpretation and clinical findings.

Cytopathology is uniquely positioned as a crucial member of the multidisciplinary team charged with triaging patient cytologic samples for appropriate, clinically relevant molecular diagnostic assays. This is predicated on the indispensable cytomorphologic evaluation for adequacy and diagnosis with integration of the molecular findings. Cytopathology laboratories are advised to engage the stakeholders within these multidisciplinary teams at their respective institutions to formulate best-practice approaches.

The American Society of Cytopathology and the Papanicolaou Society of Cytopathology support and embrace this role and associated responsibilities.

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